D1

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2002 (12.09.2002)

PCT

(10) International Publication Number
WO 02/070030 A1

(51) International Patent Classification[7]: A61L 27/38, 27/20, 27/12, 27/46, 27/42

(21) International Application Number: PCT/EP02/01224

(22) International Filing Date: 6 February 2002 (06.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PD2001A000032 9 February 2001 (09.02.2001) IT

(71) Applicant *(for all designated States except US)*: **FIDIA ADVANCED BIOPOLYMERS S.R.L.** [IT/IT]; Via Ponte della Fabbrica, 3/B, I-35031 Abano Terme (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PAVESIO, Alessandra** [IT/IT]; Via San G. Barbarigo, 93, I-35141 Padova (IT). **CALLEGARO, Landranco** [IT/IT]; Via Monte Grappa, 6, I-36016 Thiene (IT).

(74) Agents: **MINOJA, Fabrizio** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/070030 A1

(54) Title: GRAFTS FOR THE REPAIR OF OSTEOCHONDRAL DEFECTS

(57) Abstract: The invention concerns the preparation and use of a biocompatible, biocomponent material constituted by: (a) a three-dimensional matrix of hyaluronic acid derivatives witle a structure containing empty spaces; (b) a porous, three-dimensional matrix constituted by a ceramic material; (c) possibly containing pharmacologically or biologically active ingredients.

GRAFTS FOR THE REPAIR OF OSTEOCHONDRAL DEFECTS

SUBJECT OF THE INVENTION

The present invention concerns the preparation and use of a biocompatible, bicomponent material constituted by:

a. a three-dimensional matrix of hyaluronic acid derivatives with a structure that contains empty spaces created by communicating pores and/or a tangle of fine fibres or microfibres and;

b. a porous, three-dimensional matrix constituted by a ceramic material or by a composite material containing at least one ceramic material;

c. and possibly containing pharmacologically or biologically active ingredients;

for the preparation of engineered, osteochondral grafts containing a cartilage part and a bony part, separate but structurally integrated, using osteochondrogenic cells and for the repair/regeneration in vivo of structurally integrated osteochondral tissue.

BACKGROUND OF THE INVENTION

The loss of osteochondral tissue from the joints following extensive arthritis, necrosis or as a result of a trauma or the removal of a tumour requires reconstructive surgery to repair the defect.

The treatment of such defects by means of joint replacements is limited by the fact that the non-biological materials used are prone to wear and tear, and that the replacements themselves may be difficult to move.

Alternatively, it is possible to resort to autologous, allogeneic or xenogeneic bone grafts. The ideal way of substituting bone is by grafting autologous tissue, which has a better osteogenic capacity than allografts and xenografts, as shown by the presence, during its resorption, of mesenchymal

cells that differentiate into osteogenic and chondrogenic cell lines (K. L. B. Brown et al., Surg. 1982, 64A, 270-279). Moreover, allografts and xenografts carry antigens of histocompatibility, making it necessary to administer immunosuppressive treatment to the patient, while autografts do not trigger any immunological response. Besides this, there is the possibility that allografts and xenografts may transmit viruses to the recipient, such as HIV, hepatitis or BSE.

Since bone autografts cause a second trauma to the patient when the graft is taken from the donor site, and because of the paucity of available tissue, the use of this procedure is rather limited.

Orthopaedic research has long been focused on the study of new, artificial materials suitable for osteochondral grafts which would reduce or eliminate the need to resort to tissue grafts.

In order to meet requirements, a material must be biocompatible, bioresorbable at a rate that is comparable to bone growth, able to bear load, be easy to sterilise and process, be osteoactive, that is, it must be an osteoinductor, to induce mesenchymal cell differentiation into bone progenitor cells, and an osteoconductor, to enable the growth of bone within the graft.

The techniques reported in the literature for bone regeneration refer to the use of ceramics, polymers, composite materialss and bioactive molecules.

The fact that, from a chemical and structural point of view, calcium phosphates are similar to the mineral part of bone that is mainly constituted by biological hydroxyapatites, has promoted the use of ceramics as biomaterials to induce osteogenesis.

The ceramics most commonly used are beta-tricalcium phosphate (TCP) and synthetic hydroxyapatite. They have proved to be

osteoconductors, thanks to their porosity that favours cell colonisation and bone growth.

Studies conducted with subcutaneous implants in syngeneic rats have shown that the combination of bone marrow cells and porous ceramics promote osteogenesis, with the formation of new bone within the pores. Moreover, small, isolated areas of cartilage without any appreciable endochondral ossification have been observed (H. Ohgushi et al., J. Orthop. Res., 1989, 4, 568-578).

The widespread use of polymers is explained by the possibility of obtaining different compositions and structures able to satisfy the requirements of the specific applications as well as the property of biodegradation. It is known that polylactic acid, polyglycolic acid and copolymers or derivatives thereof can be used, in various forms, as biomaterials for the growth of osteocytes. The main disadvantage of using such scaffolds is represented by the immune response directed against the implanted material.

In order to create a biomaterial that is satisfactory both from a mechanical and biological point of view, various composite materials formed by mixtures of polymers and calcium phosphates have been investigated.

It is also known that scaffolds containing at least one hyaluronic acid derivative can be used as biomaterials for tissue growth.

Hyaluronic acid is a polysaccharide ether composed of alternate residues of D-glucuronic acid and N-acetyl-glucosamine. It is a linear polymer chain with a molecular weight that varies between 50,000 and 13,000,000 Da, depending on its source and on the methods of preparation and determination that are used. It is present in nature in the pericellular gels, in the fundamental substance of the connective tissue of vertebrate

organisms of which it is the main component, in the synovial fluid of joints, in the vitreous humor, in human umbilical cord and in rooster combs.

Hyaluronic acid plays a vital role in many biological processes such as hydration, proteoglycan organisation, cell differentiation, proliferation and angiogenesis (J. Aiger et al., L. Biomed. Mater. Res. 1998, 42, 172-181).

It is also known that hyaluronic acid fractions can be used to enhance tissue repair, to substitute the intraocular fluid, or they can be administered by the intra-articular route to treat joint pathologies, as described in European patents No.s 0138572 and 0535200.

Hyaluronic acid plays a fundamental role in the tissue repair process, especially in the early granulation stage, stabilising the coagulation matrix and controlling its degradation, favouring the recruitment of cells involved in the inflammatory process, such as fibroblasts and endothelial cells and, lastly, orienting the subsequent migration of epithelial cells.

It is known that the application of hyaluronic acid solutions can accelerate the tissue repair process in patients with wounds or burns. The role of hyaluronic acid in the various phases of the tissue repair process has been described by the construction of a theoretical model by P. H. Weigel et al., J. Theor. Biol., 119:219, 1986.

The use of low-molecular-weight fractions of hyaluronic acid and the autocrosslinked derivatives thereof is also known in the preparation of pharmaceutical compounds that are osteoinductors (WO 93/20827).

The total or partial esters of hyaluronic acid (HYAFF®) and its autocrosslinked derivatives (ACP®) are known, as is their use in the pharmaceutical and cosmetic fields and in that of biodegradable materials (US patents 4,851,521; 4,965,353 and 5,676,964).

In particular, patent application No. WO 93/20858 describes binding solutions and pastes containing hyaluronic acid and/or the ester derivatives

thereof used as bone fillers in surgery.

Lastly, esters of hyaluronic acid have been processed in the form of non-woven structures according to the process described in US patent 5,520,916.

Hyaluronic acid derivatives in three-dimensional form and, in particular, partial and total esters of hyaluronic acid (HYAFF®) processed in the form of non-woven tissues have been used as scaffolds in the preparation of biological materials containing cells and/or products generated from such cells.

For example, it has recently investigated the possibility of using the benzyl ester of hyaluronic acid (HYAFF®-11) as a scaffold, in a form of a non-woven fiber structure, for the culture of human chondrocytes in tissue-engineering procedures of cartilage reconstruction. In these 3D cultures, chondrocytes were able to produce hyaline cartilage-specific matrix molecules like collagen type II or proteoglycans, (J. Aiger et al., L. Biomed. Mater. Res. 1998, 42, 172-181).

We can, moreover, mention patent application No. WO 97/18842 that describes a material containing:

a. an efficient culture of autologous or homologous stem cells from bone marrow, partially or completely differentiated into cells of a specific connective tissue, containing moreover the matrix secreted by said cells, or alternatively,

a'. the extra-cellular matrix secreted by completely or partially differentiated bone marrow stem cells or, alternatively, by mature cells of the tissue;

b. a three-dimensional matrix consisting of hyaluronic acid derivatives and, in particular, partial or total esters (HYAFF®).

Hyaluronic acid derivatives in the form of sponges (HYAFF®-11

sponge, made of benzyl ester of hyaluronic acid, and ACP® sponge, made of cross-linked hyaluronic acid) used in combination with mesenchymal cells, implanted subcutaneously in nude mice, have exhibited a better osteogenic and chondrogenic capacity in terms of quantity of tissue formed, than porous ceramics (L. A. Solchaga et al., J. Orthop. Res., 17, 1999, 205-213).

In another experiment, the above said two hyaluronan derivatives-based biomaterials were tested for their ability to enhance the natural healing response of the articular cartilage for self-repair. The introduction of these polymers into osteochondral defects (made on the femoral condyles of rabbits) provides an appropriate scaffolding for the reparative process: in fact, the defects treated with HYAFF®-11 and ACP® sponges exhibited good bone fill and the surface of the condyles was mainly constituted of hyaline cartilage. (L. A. Solchaga et al., J. Orthop. Res., 18, 2000, 773-780).

It has proved extremely important to guarantee the development of a stable interface between the joint cartilage and underlying bone structure when repairing osteochondral defects.

Composite materials constituted by engineered cartilage stitched over an osteoconductor biomaterial scaffold, for the regeneration of osteochondral tissue have been implanted in defects created in rabbit joints. Six months later, it was possible to observe the remodelling of the composite material into an osteochondral tissue structurally similar to the natural variety, with a clear tidemark between the cartilage and the subchondral bone (D. Schaefer et al., 4th Annual Meeting of the Orthopaedic Research Society, 2000).

Therefore, although the use of hyaluronic acid derivatives and ceramic materials was already known both for the regeneration/repair of cartilage and bone and as scaffolds for the culture of differentiated and non-

differentiated cells, and that an expert in the field could, consequently, have deduced that it was possible to use either of these materials to make osteochondral grafts, it could not have been foreseen, as demonstrated by the present invention, that once the two materials had been coupled, it would be possible to obtain separate formations of cartilage and bone that are structurally integrated but with no penetration between the tissues, like natural osteochondral tissue.

DETAILED DESCRIPTION OF THE INVENTION

The present invention concerns the preparation and use of a biocompatible, bicomponent material constituted by:

a. a three-dimensional matrix of hyaluronic acid derivatives, with a structure containing spaces created by communicating pores and/or a tangle of fine fibres or microfibres;

b. a porous three-dimensional matrix, constituted by a ceramic material or a composite material containing at least one ceramic material;

for the preparation of structurally integrated, engineered, osteochondral grafts using osteochondrogenic cells and for the regeneration/repair of osteochondral tissue in vivo.

A further subject of the present invention is the use of the composite biomaterial as described above for the repair/regeneration of osteochondral defects, obtained by the coupling of a hyaluronic acid derivative and another material, that may be a ceramic or a derivative thereof or another component with osteoactive properties. It is possible to use said composite material in association with osteogenic cells for the preparation of engineered, osteochondral tissue.

The hyaluronic acid derivatives used in the three-dimensional scaffold (A) are chosen from the following group:

- hyaluronic acid esters wherein part or all of the carboxy functions are

esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic series (EP 0216453 B1);

- autocrosslinked esters of hyaluronic acid wherein part or all of the carboxy groups are esterified with alcoholic functions of the same polysaccharide chain or of other chains (EP 0341745 B1);

- autocrosslinked composite materials of hyaluronic acid wherein part or all of the carboxy groups are esterified with polyalcohols of the aliphatic, aromatic, arylaliphatic or heterocyclic series, generating crosslinking by means of spacer chains (EP 0265116 B1);

- hemiesters of succinic acid or heavy metal salts of the hemiester of succinic acid with hyaluronic acid or with partial or total esters of hyaluronic acid (WO 96/357207);

- O-sulphated derivatives (WO 95/25751) or N-sulphated derivatives (WO 98/45335);

- amides of hyaluronic acid or of its derivatives (WO 00/01733).

The hyaluronic acid derivatives may be in the form of non-woven fabrics, membranes, sponges. Said hyaluronic acid derivatives may in turn include pharmacologically or biologically active substances.

The porous three-dimensional matrix, as per point B, may be constituted by:

- hydroxyapatite or other calcium phosphates, including tricalcium phosphate;

- calcium sulphates;

- a composite material constituted by hydroxyapatite and tricalcium phosphate;

- a composite material constituted by:

a) hydroxyapatite or other calcium phosphates or calcium sulphates;

b) other biocompatible ceramics, metal particles or polymers;

- a ceramic material coated with hydroxyapatite or other calcium phosphates, including tricalcium phosphate;

- bioactive glass.

Said materials may in turn include pharmacologically or biologically active substances.

The pharmacologically active substances of choice are antibiotics, disinfectants and antiseptics, antiviral, antimicrobic and antifungal agents, nonsteroid and steroid anti-inflammatory drugs, cytostatic, cytotoxic, anaesthetic and anticancer agents.

Of the biologically active substances, we should mention those that favour the adhesion of cells to the biomaterial, such as fibronectin, "RGD" or integrin sequences, growth factors such as "transforming growth factor beta" (TGF-beta), insulin-like growth factor (IGF), epidermal growth factor (EGF), acid or basic fibroblast growht factor (aFBF or bFBF), hepatocyte growth factor (HGF), keratinocyte growth factor, bone morphogenic proteins (BMPs) such as BMP-1, BMP-2, BMP-3, BMP-4, BMP-5, BMP-6, osteogenic proteins (Ops) wuch as OP-1, OP-2, OP-3, growth proteins, vitamins such as vitamins C, D, E and other natural substances such as glucosamine sulphate.

The various three-dimensional matrices that constitute the components of the composite biomaterials for osteochondral grafts are assembled with the aid of a natural, synthetic or semi-synthetic, biocompatible, preferably absorbable glue, that is sufficiently adhesive. For example, it is possible to use a fibrin glue of biological origin that is widely used in clinical practice and has proved to be suitable and effective for this purpose.

Alternatively, should the materials of the various three-dimensional matrices that constitute the components of the composite biomaterial allow it, it is possible to join them by suture or with items such as screws or nails,

as used in health care and surgery.

Once the composite, bicomponent material is ready, it can be grafted into the site of the defect as it is, without any cellular components or products thereof, to act as a supporting structure in the regeneration/repair of osteochondral tissue in vivo.

Alternatively, said composite material may contain suitably inoculated differentiated or non-differentiated cells and can be used as a scaffold in which to grow said cells before grafting.

Moreover, it is possible to assemble the aforesaid two components after osteochondrogenic cells have been inoculated into them. In particular, the three-dimensional scaffold constituted by a hyaluronic acid derivative may be loaded with progenitor cells or with mature chondrocytes in order to repair/regenerate the cartilage tissue. The three-dimensional, porous matrix can be used as a scaffold for the culture of mesenchymal cells or mature bone cells in order to repair/regenerate bone.

Example of the preparation of the osteochondral graft

Cell culture

It is known from the literature that progenitor, osteochondral cells known as mesenchymal stem cells (MSCs) also differentiate into chondrocytes and osteocytes.

In the case of the present invention, mesenchymal stem cells were isolated from rat bone marrow and grown and expanded according to the protocol that appears in the literature (Lennon et al., In vitro Cell Devel. Biolo., 1996, 32, 602-611).

When the primary cells have reached confluence, they are detached from the dish by enzymatic treatment and expanded to obtain different preparations for use in different conditions.

To favour the differentiation of the mesenchymal stem cells along the

chondrocyte line, they are grown in the presence of TGF-beta 1 at 10 ng/ml. To favour the differentiation of mesenchymal stem cells into osteocytes, they are supplemented with, for instance, 100 nM dexamethasone, 10 mM beta-glycerophosphate and 0.05 mM of ascorbic acid-2-phosphate (osteogenic supplement).

The culture medium is changed every 3-4 days.

Construction of the composite graft in vitro

Before they reach confluence, the cells are detached from the dish by enzymatic treatment, counted, suspended in a serum-free medium at a concentration of $5x10^6$/ml, and loaded into the materials, for example by the bland vacuum technique.

MSCs exposed to TGF-beta 1 were loaded into a sponge made of a hyaluronan derivative (HYAFF®-11) for the construction of the cartilage component of the composite graft, and MSCs exposed to osteogenic supplement were loaded into a porous calcium phosphate ceramic component for bone formation. Cell-loaded HYAFF®-11 sponge and ceramic were assembled and joined together with fibrin glue to form a composite osteochondral graft. Said graft is incubated at 37°C for 30 minutes and then grafted subcutaneously into the backs of syngeneic rats in special pockets made with a blunt instrument.

The animals are sacrificed three to six weeks later and the material is histologically processed.

Results

The composite material remains in one piece after in vivo grafting and is encapsulated in a thin layer of fibrous tissue that can easily be removed. There are no signs of the cartilage part and the bone part having become separated in any way.

Three weeks later, fibrocartilage tissue can be seen in the empty spaces

of the matrix based on hyaluronic acid derivatives, especially in the peripheral part of the material. After six weeks (fig. 1), well-organised fibrocartilage is distributed through the material, that is partially absorbed. The chondrocytes are located in lacunae surrounded by extracellular matrix. In the extracellular matrix of the cartilage tissue, only type II cartilage can be seen.

Three weeks after grafting, bone tissue can be seen in the pores in the ceramic material, and the quantity of this tissue increases with time.

The fibrin glue has been completely absorbed after six weeks.

The separate formation of cartilage and bone can be seen in the two materials: neither the bone tissue nor the cartilage crosses the tidemark between the two materials. At the same time, the two materials form a structurally integrated composite material thanks to the presence of fibrous tissue and collagen fibres that do cross the tidemark.

This suggests that it is possible to construct a composite graft of engineered, osteochondral tissue using different materials that act as a scaffold for the chondrogenic and osteogenic differentiation of mesenchymal cells, thanks to their specific chondro-inductive and osteo-inductive potentials.

The invention being thus described, it is clear that this experiment can be modified in various ways with regard to the working conditions and methods. Such modifications are not to be considered as divergences from the spirit and purpose of the invention, and any modification that would be evident to an expert in the field comes within the scope of the following claims.

CLAIMS

1. A biocompatible, bicomponent material constituted by:
   a. a three-dimensional scaffold of hyaluronic acid derivatives, with a structure enclosing empty spaces created by communicating pores and/or a tangle of fine fibres or microfibres with or without cells and;
   b. a porous, three-dimensional scaffold constituted by a ceramic material or by a composite material containing at least one ceramic material with or without cells;
   c. and optionally containing pharmacologically or biologically active substances,

for the preparation of engineered osteochondral grafts, containing a cartilage part and a bone part that are separate but structurally integrated, by osteochondrogenic cells and for the repair/regeneration of structurally integrated osteochondral tissue in vivo.

2. A biocompatible, bicomponent material according to claim 1, wherein the three-dimensional scaffold (A) is constituted by hyaluronic acid esters wherein part or all of the carboxy functions are esterified with alcohols of the aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic series.

3. A biocompatible, bicomponent material according to claim 1, wherein the three-dimensional scaffold (A) is constituted by autocrosslinked esters of hyaluronic acid, wherein part or all of the carboxy groups are esterified with the alcoholic functions of the same polysaccharide chain or of other chains.

4. A biocompatible, bicomponent material according to claim 1, wherein the three-dimensional scaffold (A) is constituted by crosslinked compounds of hyaluronic acid wherein part or all of the carboxy groups are esterified

with polyalcohols of the aliphatic, aromatic, arylaliphatic, heterocyclic series, generating crosslinking by means of spacer chains.

5. A biocompatible, bicomponent material according to claim 1, wherein the three-dimensional scaffold (A) is constituted by hemiesters of succinic acid or heavy metal salts of the hemiester of succinic acid with hyaluronic acid or with partial or total esters of hyaluronic acid.

6. A biocompatible, bicomponent material according to claim 1, wherein the three-dimensional scaffold (A) is constituted by sulphated derivatives of hyaluronic acid.

7. A biocompatible, bicomponent material according to claim 1, wherein the three-dimensional scaffold (A) is constituted by amides of hyaluronic acid or the derivatives thereof.

8. A biocompatible, bicomponent material according to claim 1, wherein the porous, three-dimensional scaffold (B) is constituted by hydroxyapatite or other calcium phosphates, including tricalcium phosphate, calcium sulphates, a composite material constituted by hydroxyapaptite and tricalcium phosphate, a composite material constituted by hydroxyapatite or other calcium phosphates or calcium sulphates and other ceramics, biocompatible metal particles or polymers, a ceramic material coated with hydroxyapatite or other calcium phopshates, including tricalcium phospate, bioactive glass.

9. A biocompatible, bicomponent material according to claim 1, wherein the pharmacologically active ingredients are chosen from the group formed by antibiotics, disinfectants and antiseptics, antiviral, antimicrobial and antifungal agents, nonsteroid and steroid anti-inflammatory drugs, cytostatic, cytotoxic, anaesthetic and anticancer agents.

10. A biocompatible, bicomponent material according to claim 1, wherein the biologically active ingredients are chosen from the group formed by

substances that favour the adhesion of cells to the biomaterial, growth factors, bone morphogenic proteins, osteogenic proteins, growth hormones, vitamins and other natural substances such as glucosamine sulphate.

11. A biocompatible, bicomponent material according to claim 1, wherein the components (A) and (B) are made in the form of non-woven fabrics, membranes, sponges.

12. A biocompatible, bicomponent material according to claim 1, wherein the osteo-chondrogenic cells are chosen from the group formed by osteocytes, chondrocytes, bone marrow, mesenchymal stem cells.

13. A biocompatible, bicomponent material according to claim 12, wherein the cells are autologous, allologous, xenologous.

14. A process for the preparation of engineered osteochondral grafts according to claim 1 involving the following steps:

- the preparation of the three-dimensional scaffold based on hyaluronic acid derivatives (A);
- the preparation of the porous three-dimensional scaffold based on ceramic materials (B);
- the coupling of scaffolds (A) and (B) with adhesive materials and/or suture or by securing them with articles used in health care and surgery;
- the culture of osteochondrogenic cells on the biocompatible, bicomponent material in vitro;
- the grafting of the biocompatible, bicomponent material with cells, and optionally one or more pharmacologically or biologically active ingredients, into the osteochondral defect.

15. A process for the preparation of engineered, osteochondral grafts according to claim 1 involving the following steps:

- the preparation of three-dimensional scaffolds constituted by

hyaluronic acid derivatives (A);

- the preparation of porous, three-dimensional scaffolds constituted by ceramic materials (B);
- the coupling of scaffolds (A) and (B) with adhesive material and/or suture or by securing them with articles used in health care and surgery;
- the seeding and possible culture of osteo-chondrogenic cells on the biocompatible, bicomponent material in vitro;
- the grafting of the biocompatible, bicomponent biomaterial with cells in a site other than the osteochondral defect to be treated that is able to generate osteochondral tissue;
- the grafting of the same, possibly in association with one or more pharmacologically or biologically active ingredients, into the osteochondral defect.

16. A process for the preparation of osteochondral grafts according to claim 1 involving the following steps:

- the preparation of the three-dimensional scaffold based on hyaluronic acid derivatives (A);
- the preparation of the porous, three-dimensional scaffold based on ceramic materials (B);
- the coupling of the scaffolds (A) and (B) with adhesive materials and/or suture or by securing them with articles used in health care and surgery;
- the grafting of the biocompatible, bicomponent biomaterial without cells, optionally loaded with one or more pharmacologically or biologically active ingredient, into the osteochondral defect.

17. A process for the preparation of engineered, osteochondral grafts according to claim 1 involving the following steps:

- the preparation of the three-dimensional scaffold based on hyaluronic acid derivatives (A) and the seeding of differentiated or non-differentiated mesenchymal stem cells or bone marrow cells;
- the preparation of the porous three-dimensional scaffold based on ceramic materials (B) and the seeding of differentiated or non-differentiated mesenchymal stem cells or bone marrow cells;
- the coupling of the scaffolds (A) and (B) with adhesive materials and/or suture or by securing them with articles used in health care and surgery and culture;
- the grafting of the biocompatible, bicomponent biomaterial with cells, possibly in association with one or more pharmacologically or biologically active ingredients, into the osteochondral defect.

18. The use of engineered, osteochondral grafts according to the previous claims for the repair/regeneration of osteochondral defects.

19. The use of engineered, osteochondral grafts according to the previous claims in the form of non-woven fabrics, membranes, sponges.

20. The use of engineered, osteochondral grafts according to claim 19, in which the non-woven fabric is made of benzyl ester of hyaluronic acid (HYAFF®11).

21. The use of engineered, osteochondral grafts according to claim 19, in which the non-woven fabric is made of cross-linked hyaluronic acid (ACP®).

22. The use of engineered, osteochondral grafts according to claim 19, in which the sponge is made of benzyl ester of hyaluronic acid (HYAFF®11).

23. The use of engineered, osteochondral grafts according to claim 19, in which the sponge is made of cross-linked hyaluronic acid (ACP®).

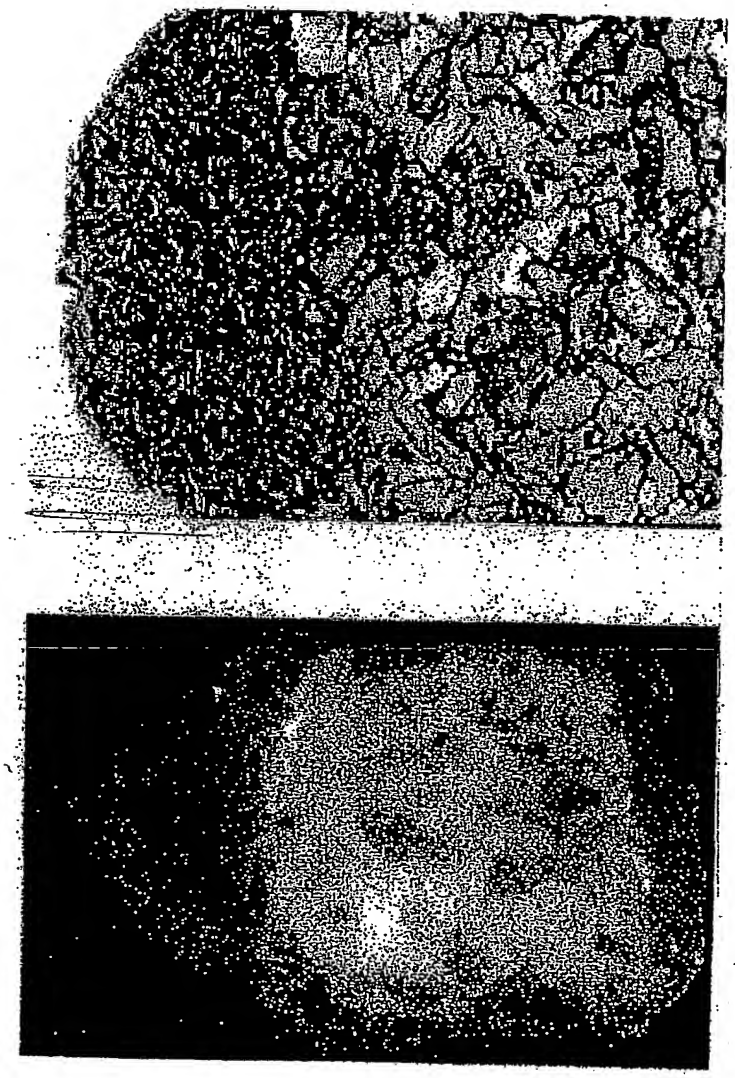

Figure 1a

Figure 1b

Figure 1: gross appearance (Fig. 1a) and histology section (Fig. 1b; stained with toluidine blue; x 4) of a sample taken 6 weeks after grafting.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/01224

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/38 A61L27/20 A61L27/12 A61L27/46 A61L27/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
INSPEC, CHEM ABS Data, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GAO, JIZONG ET AL: "Tissue-engineered fabrication of an osteochondral composite graft using rat bone marrow-derived mesenchymal stem cells" TISSUE ENGINEERING (2001), 7(4), 363-371 , August 2001 (2001-08), XP008005883 the whole document	1-23
Y	WO 93 20858 A (CALLEGARO LANFRANCO ;FIDIA SPA (IT); ROMEO AURELIO (IT); DORIGATTI) 28 October 1993 (1993-10-28) claims; examples	1-23

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 2002	24/07/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	ESPINOSA, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	BAKOS D ET AL: "Hydroxyapatite-collagen-hyaluronic acid composite" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 20, no. 2, January 1999 (1999-01), pages 191-195, XP004168893 ISSN: 0142-9612 the whole document	1-23
Y	WO 94 17837 A (CALLEGARO LANFRANCO ;DORIGATTI FRANCO (IT); FIDIA ADVANCED BIOPOLY) 18 August 1994 (1994-08-18) claims; examples 1-7	1-23
A	WO 99 61080 A (CALLEGARO LANFRANCO ;DONA MASSIMO (IT); FIDIA ADVANCED BIOPOLYMERS) 2 December 1999 (1999-12-02) claims	1-23
A	WO 99 65534 A (CALLEGARO LANFRANCO ;PASTORELLO ANDREA (IT); RADICE MARCO (IT); FI) 23 December 1999 (1999-12-23) claims; examples	1-23
A	BURG K J L ET AL: "Biomaterial developments for bone tissue engineering" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 21, no. 23, 1 December 2000 (2000-12-01), pages 2347-2359, XP004216904 ISSN: 0142-9612 page 2355	1-23
A	WO 00 61675 A (CALLEGARO LANFRANCO ;PALLADO PAOLO (IT); BAGGIO MARCO (IT); DALLE) 19 October 2000 (2000-10-19) claims	1-23
A	WO 00 37124 A (CALLEGARO LANFRANCO ;PASTORELLO ANDREA (IT); RADICE MARCO (IT); FI) 29 June 2000 (2000-06-29) claims; examples	1-23
A	WO 00 01733 A (BELLINI DAVIDE ;FIDIA ADVANCED BIOPOLYMERS SRL (IT); TOPAI ALESSAN) 13 January 2000 (2000-01-13) claims; examples	1-23

NSDOCID: <WO_____02070030A1_I_>

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/01224

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9320858	A	28-10-1993	IT	1259090 B	11-03-1996
			CA	2118218 A1	28-10-1993
			WO	9320858 A1	28-10-1993
			EP	1142595 A2	10-10-2001
			EP	0637254 A1	08-02-1995
			JP	7505643 T	22-06-1995
			US	2001053938 A1	20-12-2001
WO 9417837	A	18-08-1994	IT	1263316 B	05-08-1996
			AU	681873 B2	11-09-1997
			AU	6108894 A	29-08-1994
			CA	2155319 A1	18-08-1994
			WO	9417837 A1	18-08-1994
			EP	0683679 A1	29-11-1995
			JP	8506255 T	09-07-1996
			US	5658582 A	19-08-1997
WO 9961080	A	02-12-1999	IT	PD980131 A1	29-11-1999
			IT	1302535 B1	05-09-2000
			AU	748303 B2	30-05-2002
			AU	4368099 A	13-12-1999
			WO	9961080 A1	02-12-1999
			EP	1085917 A1	28-03-2001
			JP	2002516154 T	04-06-2002
WO 9965534	A	23-12-1999	IT	PD980149 A1	17-12-1999
			AU	4611599 A	05-01-2000
			WO	9965534 A1	23-12-1999
			EP	1087797 A1	04-04-2001
WO 0061675	A	19-10-2000	IT	PD990071 A1	09-10-2000
			AU	4912600 A	14-11-2000
			WO	0061675 A1	19-10-2000
			EP	1169387 A1	09-01-2002
WO 0037124	A	29-06-2000	IT	1302534 B1	05-09-2000
			AU	1791600 A	12-07-2000
			EP	1140240 A1	10-10-2001
			WO	0037124 A1	29-06-2000
			US	2002076810 A1	20-06-2002
WO 0001733	A	13-01-2000	IT	PD980169 A1	07-01-2000
			AU	4639799 A	24-01-2000
			EP	1095064 A1	02-05-2001
			WO	0001733 A1	13-01-2000

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2002 (12.09.2002)

PCT

(10) International Publication Number
WO 02/070030 A1

(51) International Patent Classification[7]: A61L 27/38, 27/20, 27/12, 27/46, 27/42

(21) International Application Number: PCT/EP02/01224

(22) International Filing Date: 6 February 2002 (06.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PD2001A000032 9 February 2001 (09.02.2001) IT

(71) Applicant *(for all designated States except US)*: **FIDIA ADVANCED BIOPOLYMERS S.R.L.** [IT/IT]; Via Ponte della Fabbrica, 3/B, I-35031 Abano Terme (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PAVESIO, Alessandra** [IT/IT]; Riviera Paleocapa, 9, I-35141 Padova (IT). **CALLEGARO, Lanfranco** [IT/IT]; Via Monte Grappa, 6, I-36016 Thiene (IT).

(74) Agents: **MINOJA, Fabrizio** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(48) Date of publication of this corrected version:
5 December 2002

(15) Information about Correction:
see PCT Gazette No. 49/2002 of 5 December 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: GRAFTS FOR THE REPAIR OF OSTEOCHONDRAL DEFECTS

(57) Abstract: The invention concerns the preparation and use of a biocompatible, biocomponent material constituted by: (a) a three-dimensional matrix of hyaluronic acid derivatives witle a structure containing empty spaces; (b) a porous, three-dimensional matrix constituted by a ceramic material; (c) possibly containing pharmacologically or biologically active ingredients.

WO 02/070030 A1